

GERMARIAL DIFFERENCES AND THE PRODUCTION OF APHID TYPES *

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INTRODUCTION

If germaria exercise any control over the development of differential characters in female aphids (Lawson, 1939; 1940) it is possible that they would give evidence of this control by exhibiting structural peculiarities correlated with the production of specific aphid types. To investigate this possibility the germaria of parthenogenetic females producing different aphid types were compared.

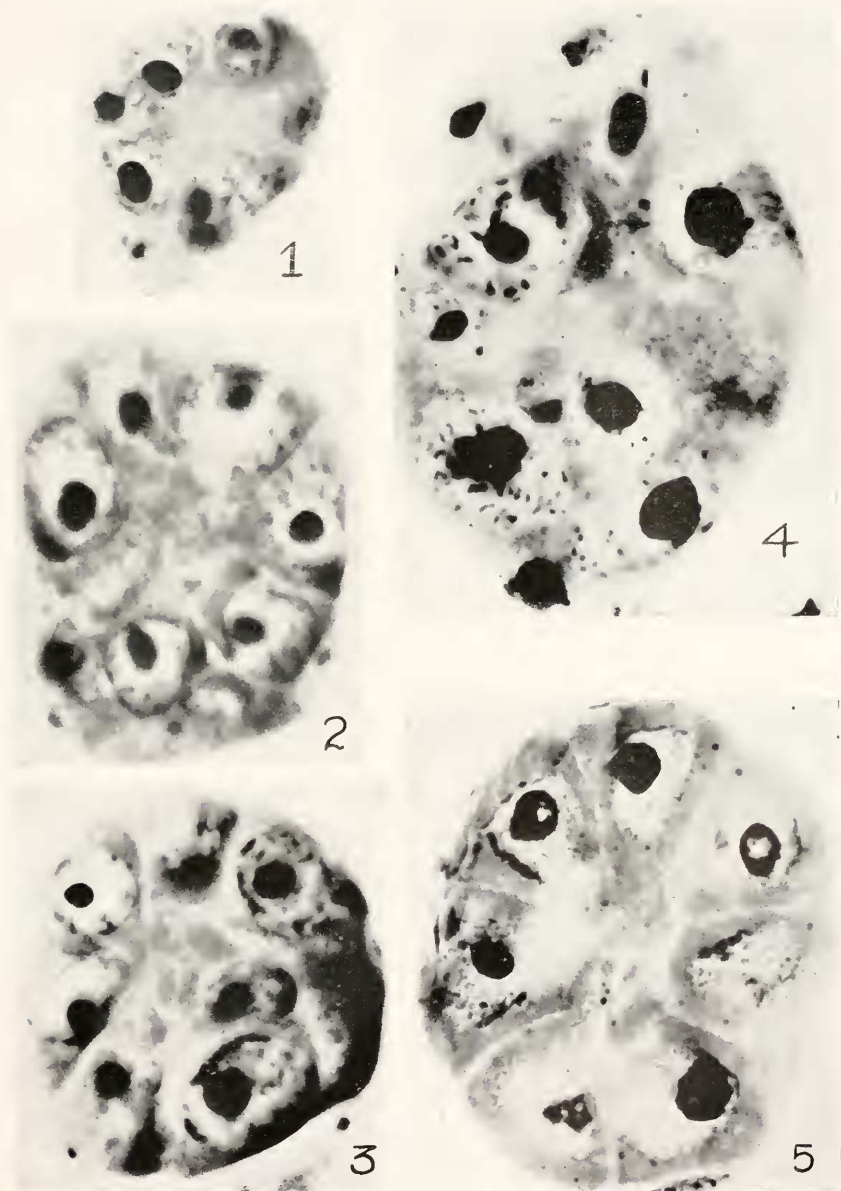
THE GERMARIA

Each adult germarium contains two types of cells, nurse cells and germ cells. The nurse cells are larger than the germ cells and make up the bulk of the germarium, so if the germarium controls development it is possible that this control stems from the nurse cells. Their prominence in the germarium at least gives them first choice of the parts to be tested, so in this study the nurse cells only are compared.

The nurse cells of all parthenogenetic germaria are essentially alike (Figs. 1, 2, 3, 4). Each nurse cell is roughly pyramidal in shape (triangular in section) with the base at the periphery of the germarium and the apex in the center. The nucleus lies near the base of the pyramid and is covered on its outer edge and sides by a thin layer of cytoplasm. On the inner border of the nucleus the cytoplasm is thicker and extends inward toward the center of the germarium forming the apex of the pyramid. The cytoplasm seldom forms a sharp point in the center, for here it blends with the secreted substance found in the center of all germaria. The exact line of demarcation between cytoplasm and secreted material is difficult to see. The nuclei of all nurse cells are relatively large and each contains a large elliptical nucleolus and chromatin in the form of thin rods or prophase strands that are interconnected by a fine threadlike network.

In comparing the germaria of different aphid types, structural differences were sought that would serve to differentiate among them. Of several possible structural differences only one stands out with any consistency. This is a size difference. To test the reality of this apparent difference measurements were made and compared of the entire germarium and of individual nuclei within the nurse cells.

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FIGURES 1-5. Cross-sections of adult aphid germaria. Figure 1. Winged parthenogenetic female producing gametic embryos (1455 \times). Figure 2. Winged parthenogenetic female producing parthenogenetic female embryos (1455 \times). Figure 3. Wingless parthenogenetic female producing parthenogenetic female embryos (1455 \times). Figure 4. Wingless parthenogenetic female producing male embryos (1455 \times). Figure 5. Adult gametic female (675 \times).

As each germarium is approximately spherical in shape its center cross section is circular or elliptical. The diameters of this cross-section were measured in micra and the area computed and this figure used to represent the size of the germarium. A better method of comparing the germaria would be to compare volumes. In order to calculate the volume of any one germarium it is necessary to have three diameters because very few of the germaria are perfect spheres. Two of these are easily measured on the center cross section. The third can be gotten by counting the number of cross sections of the germarium. However, no great reliance can be placed on a measurement arrived at in this manner. Each cross section is ten micra in thickness except the first and the last. These two vary from a fraction of one to ten micra, and as the actual thickness cannot be determined the third diameter has a possible error of twenty micra. Because of this error no confidence can be placed in the calculated volumes and it seems best to restrict the comparisons to the more accurately measureable center areas of the germaria. An occasional irregularity in the circumference of the cross sections introduces a source of error which is probably not great enough to discount major size differences, but may affect the results in comparison of minor differences.

Each aphid has nine or ten germaria and all of these that could be measured accurately were measured and all measurements from one type of aphid were grouped and treated statistically.

The means and standard deviations of the area of the center cross section of the adult germaria are given in Table I. The germaria of the male-producing

TABLE I

A comparison of the areas in square micra of germarial center section

Type of female	Contained embryos	n	Mean	Standard deviation
1. wingless parth.	males	103	1474±42	422±29
2. wingless parth.	parth. females	172	731±14	190±10
3. winged parth.	parth. females	126	599±16	178±11
4. winged parth.	gamic females	127	567±10	115±7

wingless parthenogenetic females (Fig. 4) are larger than those of the wingless females producing parthenogenetic females (Fig. 3) and these in turn are larger than the germaria of winged females (Figs. 1 and 3). The differences between the means are statistically significant for all except the two winged types.

A difference between two means is considered significant when it is at least twice the standard error of the difference between means.

The Nurse Cell Nuclei

The nuclei of the nurse cells also were measured and compared. All the nuclei in any one germarium were not measured, but only those that were spherical. Many of the nuclei formed long ellipses or varied from the spherical unevenly. These nuclei were rejected in order to reduce the error of measurement and also to reduce the labor. If spherical nuclei only are used, one measurement, the

diameter, is sufficient; and from this the volume can be calculated. This selection may introduce an error in the results if the shape of the fixed nucleus is correlated with its size, which is unlikely; or if an insufficient number of nuclei are measured in any one aphid type. It is believed that the number measured is sufficiently large to evade this source of error.

The means and standard deviations of nurse cell nuclear volumes in cubic micra are given in Tables II and III.

TABLE II

A comparison of nurse cell nuclear volume measured in cubic micra

Type of female	Embryos	n	Mean	Standard deviation
1. gamic		100	5180±314	3140±222
2. wingless parth.	males	205	953±28	402±20
3. wingless parth.	parth. females	408	326±7	140±4
4. winged parth.	parth. females	351	283±7	130±5
5. winged parth.	gamic females	200	151±5	64±3

TABLE III

Means and standard deviations in cubic micra of nurse cell nuclear volume of parthenogenetic females producing different types of parthenogenetic embryos

Type of aphid	Embryos	n	Mean	Standard deviation
1. wingless	winged and wingless	200	302±9	125±6
2. wingless	winged	208	348±10	148±7
3. winged	winged and wingless	351	283±7	130±5
4. winged	winged	198	279±8	129±6
5. winged	wingless	134	301±11	130±8

In Table II are listed the means and standard deviations of the five major aphid types. The differences in the mean nuclear volumes are all statistically significant. Thus the nurse cell nuclei of gamic female germaria (Fig. 5) are larger than any of the others, those of wingless females producing males (Fig. 4) are smaller than the gamic nuclei, but larger than any other parthenogenetic nurse cell nuclei. The wingless females producing parthenogenetic females (Fig. 3) have nurse cell nuclei that are smaller than the male-producing type but larger than those in winged females, while the winged female nurse cell nuclei are smaller than any of the others. There is also a nuclear size difference between the two types of winged females. The winged females producing gamic females (Fig. 1) have smaller nuclei than those producing parthenogenetic females (Fig. 2). In comparing Figures 1 to 5 it should be noted that the magnification of Figure 5 is approximately one-half that of Figures 1, 2, 3, 4.

The size differences shown by the nuclear measurements are in the same direction as those shown by the germarial measurements which suggest that the size of the entire germarium is due to the size of the nurse cells. One exception to this is seen in the two sets of measurements of the winged parthenogenetic females.

In comparing measurements of germarial center areas (Table I) the winged females producing parthenogenetic females and those producing gamic females are not significantly different. The means are different and direction of difference is the same as that of the nuclear size differences, but the difference is not statistically significant. In comparing the nuclear measurements of these same winged female types (Table II) a very large and significant difference appears.

One of the possible explanations is that no correlation exists between nuclear size and germarial size but rather between germarial size and nuclear (cell) number. If this is true the germaria of winged females producing gamic females should have almost twice as many nuclei as the germaria of winged females producing parthenogenetic females. A count revealed the same number in both (average 20 to 22). Another possibility is that there might be twice as much cytoplasm in each nurse cell within the gamic producing germaria, or that the material secreted by the nurse cells is excessive. These possible differences are not apparent on comparing the two types of germaria (Figs. 1 and 2) hence it is likely that there is some other explanation at present unknown. Also there remains the possibility that a difference may exist between the germarial areas of the two types of winged females (Table I) that is not shown in these calculations. The number of individuals used for computing the means of the germarial areas are one-half as many as are used in computing the means for nuclear volume of the same individuals (Table II). If n were doubled for the germarial areas a significant difference might appear.

In Table III are listed the means and standard deviations of parthenogenetic females that are producing parthenogenetic female offspring. The winged and wingless adults are classified according to whether they are producing either winged or wingless parthenogenetic female offspring or both.

The means are all about the same and none of the differences is statistically significant except for number 2 (wingless females producing winged embryos). This mean is significantly different from all in the table except number 5 (winged females producing wingless offspring). Thus except for one case no size difference is correlated with the production of parthenogenetic types and in this one case the difference is not great so it is possible that some factor other than type of offspring produced the difference.

If this interpretation is correct and there is no real size difference among the nurse cell nuclei in Table III a change must be made in the interpretation of Table II. In this table the mean nuclear sizes of number 3 (wingless parthenogenetic females producing parthenogenetic female embryos) and number 4 (winged parthenogenetic females producing parthenogenetic female embryos) are significantly different. However, the calculation of the mean of 326 ± 7 of number 3 of Table II includes the data under number 2 of Table III. If these data are eliminated from the calculations the mean becomes 302 ± 9 ($\sigma = 125 \pm 6$) and the difference disappears between this mean and that of the number 4, Table II (winged parthenogenetic females producing parthenogenetic female embryos). Thus the group of data in Table III that shows a questionable difference causes the difference between the winged and wingless parthenogenetic-producing females in Table II. Hence 3 and 4 in Table II probably are not different. There remain, however, the differences among the other types which are so large that their reality seems beyond doubt.

Germaria and Embryos of Winged-wingless Intermediates

A study of winged-wingless intermediates offers further evidence that the nurse cell nuclear volume is correlated with the type of offspring produced. In Table IV is presented a comparison of the mean nuclear volume of germarial

TABLE IV

A comparison of volumes in cubic micra of nurse cell nuclei in germaria of winged-wingless parthenogenetic female intermediates with the type of embryos contained in the vitellaria to which these germaria are attached

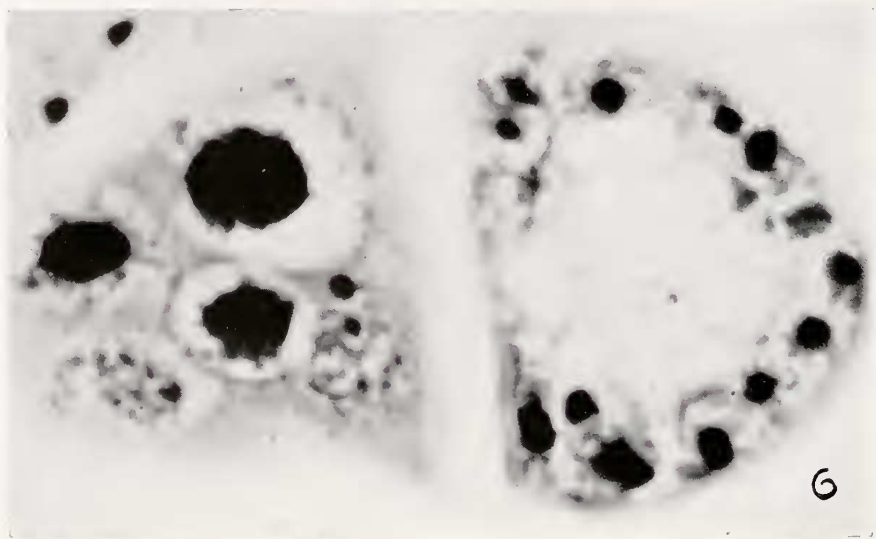
Mean nuclear volumes	Types of embryos
1. 57 ± 9	Gamic female
2. 63 ± 9	Gamic female
3. 132 ± 10	Parthenogenetic female
4. 140 ± 9	Parthenogenetic female
5. 157 ± 8	Gamic female
6. 194 ± 11	Parthenogenetic female
7. 235 ± 14	Gamic and parthenogenetic female
8. 272 ± 15	Parthenogenetic female
9. 353 ± 31	Parthenogenetic female and male
10. 381 ± 37	Parthenogenetic female and male
11. 383 ± 23	Parthenogenetic female and male
12. 383 ± 23	Parthenogenetic female
13. 486 ± 58	Parthenogenetic female and male
14. 559 ± 61	Parthenogenetic female and male
15. 732 ± 93	Male
16. 804 ± 73	Male and gamic egg
17. 930 ± 82	Male

nurse cells in individual winged-wingless intermediates and the type of embryos contained within the ovarioles of the intermediates. From this comparison it is evident that the intermediates having the smallest nuclear volume contain gamic female embryos within their ovarioles, and that as the nuclear volume becomes greater the embryos become parthenogenetic, then both parthenogenetic and male (in which the older embryos are parthenogenetic) then all male embryos and finally the intermediates having the largest nurse cell nuclear volume contain both male embryos and gamic eggs.

This correlation is not exact for intermediates 3 and 4, Table IV, contain parthenogenetic embryos while intermediate 5 has gamic embryos and also has a larger mean nuclear volume than either 3 or 4. Also intermediates 11 and 12 have the same nuclear volume, even though number 11 has both male and parthenogenetic female embryos, while number 12 has parthenogenetic embryos only. This irregularity may be due to the fact that all of the nuclei in any one intermediate could not be measured accurately, or it may be due to the effect of some unknown factor. In any case it seems reasonable to conclude that in winged-wingless intermediates the size of the nurse cell nuclei is correlated in general with the production of specific aphid types.

In one intermediate (17) there is an unusual germarium (Fig. 6) in which the nuclei are of two distinct sizes. The germarium is partly divided in half; one-half containing large nuclei ($M = 2264 \pm 390$) the other half containing small nuclei ($M = 445 \pm 53$). The appearance of two distinct sizes of nuclei within one germarium suggests that the size of any one nurse cell nucleus is determined by

some factor within the germarium and perhaps within the individual nucleus itself. What this factor might be is entirely hypothetical; however, the nuclear size variation suggests polyploidy. No chromosome counts have been made as yet, but as the nuclei of gamic female germaria are filled with small rod-shaped chromosomes and are so much larger than any of the other types of nuclei it is probable that there is more chromatin in the gamic nuclei than in the others.



FIGURES 6-7. Figure 6. Abnormal germarium of a winged-wingless intermediate showing nuclei of two sizes (675 \times). Figure 7. Degenerate body (embryo?) found in ovariole of wingless parthenogenetic female producing males (675 \times).

Are all intermediates physiologically wingless?

Shull (1940) has suggested that adult winged-wingless intermediates are not physiologically intermediate but, rather, that they are wingless having progressed during development from a winged to a wingless condition. The structural characters become fixed in an intermediate condition during the transition and remain so during the life of the aphid, but the physiological nature of the individual continues changing until it is completely wingless. As a typical winged individual produces gamic females during the gamic phase of the cycle while a wingless female produces males, the physiological nature of the intermediates was determined by examining the type of offspring produced by them. Thus, if an intermediate produced males it was judged to be physiologically wingless. If it produced gamic females it was winged. Shull concluded that all winged-wingless intermediates are physiologically wingless.

An opposite conclusion is indicated by the evidence derived from the intermediates used in this study. These intermediates produced both male and gamic female embryos. Consequently some of them were physiologically winged and some wingless.

Degeneration in male-producing wingless females

Wingless females that are producing males not only have distinctive germaria but they also have degenerating cell masses within their ovarioles. The cell masses (Fig. 7) occur in the ovarioles at any position though they were observed most frequently at the end nearest the germarium. They are elliptical in longitudinal section and circular in cross section. A vacuolated center area is usually surrounded with a rim of densely staining pycnotic cells. What the degenerating bodies are is questionable but their elliptical shape is similar to young embryos, and furthermore, the rim of cells surrounding a vacuolated non-cellular center area is typical of young male blastulae. Therefore, it is tentatively concluded that the degenerating bodies are embryos that failed to continue development and are being resorbed. Why degenerating embryos should be characteristic of male-producing wingless females remains an open question.

CONCLUSION

A correlation between the size of the germaria and their nurse cell nuclei and the type of embryos produced seems established. Whether the germaria actually control production of aphid types is still unknown.

SUMMARY

The areas of the center cross-section of adult germaria of parthenogenetic female aphids producing different types of offspring were measured and compared. From this comparison it is evident that the center cross-sections of the germaria of male-producing wingless parthenogenetic females are larger than those of wingless females producing parthenogenetic females, and these in turn are larger than the cross-section of winged female germaria. All winged females have the same cross-sectional area whether they are producing parthenogenetic or gamic females.

A comparison of the volume of the nurse cell nuclei shows that the nuclei of gamic female germaria are larger than any of the others; those of wingless females producing males are smaller than the gamic nuclei, but larger than any other parthenogenetic nurse cell nuclei. The wingless and winged females producing parthenogenetic females have nurse cell nuclei of the same size, while the nurse cell nuclei of winged females producing gamic females are the smallest of all.

A correlation of the nurse cell nuclear volume of winged-wingless intermediates with the embryos contained in the ovarioles supports the thesis that size of nuclei and type of young produced are interdependent. Those intermediates that contained gamic embryos have the smallest nuclei; those with the next in nuclear size have both parthenogenetic and male embryos. The largest contain males only or males and gamic eggs.

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